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Analyzing the Viability of Various Native Membrane Mimics for Membrane Proteins using Site-Directed Spin Labeling EPR
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Membrane proteins have become the target for the majority of drug related therapies in recent years; in contrast, there is limited information available on membrane proteins due to the difficulties of studying them in vitro. In order to study their structure and function, it is crucial to prepare suitable, native-like membrane mimics. Our studies involve KCNE1, a transmembrane protein located in the heart that modulates the activity of the KCNQ1 voltage-gated potassium channel. An important protein for proper cardiac function, mutations in the structure can lead to atrial fibrillation, long QT syndrome, and deafness. In order to assess the viability of various membrane mimics for studying membrane proteins, we have utilized site-directed spin labeling (SDSL)and electron paramagnetic resonance (EPR) spectroscopic techniques, which are well established methods of studying protein structure. The CW-EPR spectral line shape analysis was conducted on an inside probe (F56C) and outside probe (R33C) in various vesicle compositions (POPC, POPG, DMPC, DOPC, DPPC, and DOPG) in order to assess the accuracy and precision of various membrane mimics. This study will provide a path for researchers working on membrane protein EPR spectroscopic studies to select a better membrane mimetic environment.

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The Structure of the Oligomers Formed by the Caveolin Membrane Proteins
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Caveolae are omega-shaped invaginations of the plasma membrane that play essential roles in cell physiology through the regulation of trafficking and signaling. They are also involved in numerous diseases such as infections, cancer, diabetes and Alzheimer's disease. Caveolae structural integrity is largely maintained by the caveolin membrane proteins. Our recent work showed that caveolin-1 alone is able to induce budding in artificial cholesterol-containing membranes. Caveolin-1 self-assembles over 10 nm-scale membrane domains in order to perform this function. This study expands on the caveolin structure-function relationship by focusing on the aggregates formed by these proteins with a special emphasis on the role played by the C-terminus. Caveolins with both full-length and C-terminal deletions, as well as C-terminal peptides, were investigated by electron microscopy and solid-state nuclear magnetic resonance. Our results show that the caveolin C-terminus is involved in the formation of very specific aggregates that are crucial to the membrane scaffolding function of the protein.

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Expression and Purification of Human A_2b Receptor for Spectroscopic Characterization
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Adenosine A_2b receptors belong to the G protein coupled receptors family, and are implicated in asthma, regulation of cell growth, vasodilation, intestinal function, and modulation of neurosecretion. So far, there is no high-resolution structure of the protein. Thus, considering A_2b receptor as a potential therapeutic target highlights the significance of conformational characterization of the protein. We used A_2b receptor variant (b*a) containing A_2aR-based thermostable mutations (T89A, G119A, R123A, V208A, V240A), and A_2a C-terminal residues that enable expression but retain ligand-binding function of wild type A_2bR. The yeast S. cerevisiae strain BJ5464 was transformed by pITy-b*aR-His_{10} plasmid for high-level protein production. Cells growth rates were monitored by measuring the optical density (OD) at 600 nm, and b*a expression levels were analyzed by Western blot over time, indicating the maximum protein yield between OD_{600} 16-20. Following mechanical lysis of the yeast cells, b*a proteins were purified in sodium phosphate buffer (pH 7.0) containing n-dodecyl-β-D-maltoside (DDM), cholesterol hemisuccinate (CHS), and 3-(3-cholamidopropyl) dimethylammoniopropane sulfonate (CHAPS) through immobilized metal affinity chromatography. Our data with blue native polyacrylamide gel electrophoresis suggests an oligomeric state for the b*a chimera. Further, the secondary structure of b*a variant was characterized by circular dichroism spectroscopy, indicating a dominant alpha helical conformation of the protein. In conclusion, homo-oligomerization of A_2b receptors can facilitate signal transduction and improve structural stability of the complex.

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Effect of an Apoptotic Membrane raft on the Conformational and Dynamical Changes of Calreticulin
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Calreticulin (CRT) on the cell surface can mediate engulfment of apoptotic cells by interacting directly with LDL receptor-related protein (LRP1) (Cell. 123:321-34, 2005). Phosphatidyl serine (PS) lipids in the inner leaflet of the cell membrane are externalized and become exposed in cholesterol-rich domains during apoptosis and co-localized with cell surface surface CRT (Cell. 123:321-34, 2005). How the apoptotic cell membrane affects the structure and dynamics of CRT, further influencing CRT binding to LRP1 to signal apoptotic-cell clearance remain unknown. In this study, we investigated the interactions of a membrane raft in an apoptotic cell membrane with CRT and its effect on conformational and dynamical changes in CRT via atomically detailed molecular dynamics simulations. An apoptotic membrane raft membrane is modeled as a bilayer containing 1-palmitoyl-2-oleoyl-sn-glycero-3phosphocholine (POPC) lipids, cholesterol (CHOL) and palmitoyl-oleoyl phosphatidylserine (POPS) lipids (the ratio of the number of POPC lipid, POPS lipid and CHOL is: 5:1:4). The results are compared with the membrane raft without POPS lipids. POPS lipids in the membrane raft affected the microscopic and mesoscopic properties of the membrane raft. Apoptotic membrane raft resulted in the more direct interaction of the N-, partial C, and P-domains of CRT with membrane raft and more stabilized CRT conformation compared to that of CRT in a POPC-CHOL membrane raft, which could affect CRT recruitment of LRP1. Results from this study provided molecular insight into the effect of apoptotic membrane raft on CRT conformational and dynamical changes, further affecting CRT binding to LRP1 for signaling.
Keywords: membrane raft, POPS lipids, TSP1-CRT complex, apoptotic cell clearance, molecular dynamics simulations

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Binding of Halictine Antimicrobial Peptides to Model Membranes Composed of POPC:POPG Phospholipids
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Halictine antimicrobial peptides were isolated from the venom of the bee *Halictus sexcinctus*. As described in previous studies, halictinemolecules exhibit antimicrobial action against Gram-positive and -negative bacterial cells. However, they have also a considerable hemolytic activity (LC_{50} ~80 μM). Halictine 1 (HAL-1) has 12 amino acid residues (GMWSKILGHLIR-NH_2), and +3 charges. Other similar sequence, halictine 2 (HAL-2), has also 12 amino acid residues (GKWMSLLKHILK-NH_2), and +4 charges. The interaction of these peptides with model membranes composed of POPC:POPG (3:1, and 1:1 molar ratio) phospholipids were monitored using ITC and optical microscopy. For both bilayer composition, halictines were able to bind to the vesicles, resulting mainly in an exothermic process detected by ITC ($\Delta H_{pep} = -6.8$ kcal/mol, and $\Delta H_{pep} = -4.5$ kcal/mol for HAL-1; $\Delta H_{pep} = -3.4$ kcal/mol, and $\Delta H_{pep} = -5.1$ kcal/mol for HAL-2, for titrations with 3:1, and 1:1 POPC:POPG LUVs respectively). HAL-1 displayed endothermic peaks larger than HAL-2, with an additional heat signal at the end of the binding process that remains unclear. Giant unilamellar vesicles (GUVs) were monitored by optical microscopy, which allowed us to observe a peptide-induced permeabilization of these GUVs, followed by vesicle bursts, which was observed for membranes composed of 3:1, and 1:1 POPC:POPG. It could be an indirect evidence of pore formation, culminating in the total collapse of the GUVs. ITC and microscopy experiments were done with low peptide concentrations (30 μM and 20 μM, respectively), below the lethal dose (LC_{50}) of halictines, estimated as hemolytic. The results suggest that HAL-1 and HAL-2, besides the short amino acid sequences, are efficient to bind to model membranes and, thus, to promote pore formation even with low POPG ratio and also low peptide concentration.

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The Beginning of the End: Cardiolipin, Cytochrome C and the Apoptotic Trigger
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